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14. ABSTRACT

In Year 1 of this award we examined whether duraplasty after acute thoracic spinal cord injury (SCI) in a porcine model could improve long-term functional outcome after injury. The T10 spinal cord was contused with a weight drop device in which a 50g weight impact rod was dropped from a 20-cm height followed by 5 minutes compression (150g). Following injury, in n=6 animals a C6-T13 transverse dural incision was performed and a 10x1 cm artificial dural graft was sutured to the remaining dura mater in a watertight manner using a running suture of 6-0 Prolene, including the use of fibrin sealant to reinforce sutures. Control animals received an identical spinal cord contusion, however without expansile duraplasty. Behavioral and functional testing was performed weekly up to 12 weeks post-injury using the Porcine Thoracic Injury Behavior Scale and hindlimb SSEP monitoring.

Preliminary data suggest that duraplasty surgery might improve functional recovery early after SCI compared to SCI-animals without duraplasty

15. SUBJECT TERMS

Spinal Cord Swelling, Hydrostatic Pressure, Duraplasty, Spinal Cord Injury, Pressure Reactivity Index

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1 INTRODUCTION

After acute spinal cord injury (SCI) the spinal cord is frequently found to have swollen dramatically, particularly after it has been surgically decompressed. In traumatic brain injury (TBI), brain swelling and increases in intraparenchymal pressure are routinely considered in both the surgical and hemodynamic management of such patients. However, this swelling has largely been neglected in SCI, despite being consistently observed. Even after surgical decompression, such swelling may result in the cord being subjected to significant pressure, either due to constriction by the pia mater, the dura mater, or both. The physiologic consequences of this are poorly understood, and many fundamental questions remain about its impact on intraparenchymal pressure, spinal cord perfusion, and downstream metabolic responses. Determining the physiologic/biologic consequences of this swelling and how they can be mitigated to reduce secondary injury will guide the optimal clinical management of acute SCI. As an example of how swelling, increased intraparenchymal pressure, and its effects on perfusion are factored into clinical decision-making, TBI investigators have established the Pressure Reactivity Index (PRx) to identify where autoregulation remains intact and to guide optimal perfusion support based on that. The PRx has not been investigated in SCI, but given that the cord also swells and has impaired autoregulation, it is likely applicable here as well. This promising approach opens the possibility that we could individualize and optimize the hemodynamic support of acute SCI patients in order to support perfusion without exacerbating deleterious increases in intraparenchymal pressure.

2 KEYWORDS

- Spinal Cord Swelling
- Hydrostatic Pressure
- Spinal Cord Injury
- Pressure Reactivity Index
- Porcine model of SCI

3 OVERALL PROJECT SUMMARY

Human SCI is typically caused by a combination of high velocity contusion followed by sustained mechanical compression. Relieving this ‘extrinsic’ mechanical compression in a timely manner intuitively would seem to be neuroprotective and result in improved neurologic function. However, the cord itself then undergoes considerable post-decompression swelling as demonstrated in SCI-patients. The spinal cord may ultimately swell to the point where it compresses against the dura, resulting in increased intraspinal pressure at the injury site as demonstrated by Papadopoulos and colleagues (2004). Such swelling and rise in the pressure within the spinal cord has also been observed in our porcine model of SCI (Figure 1 & 2). This suggests that we can utilize this model to investigate what is happening within the spinal cord during this swelling. When the cord swells against the dura, there may be an increase in pressure and reduction in spinal cord perfusion (as suggested by Papadopoulos et al.) **Our research question is: would it be beneficial to decompress the spinal cord by opening the dura and enlarging the subarachnoid space?**

Figure 1: The swollen cord expands against the dura within hours after decompression. Prior to injury, the subarachnoid space is clearly seen between the spinal cord and dura. After SCI and within 10 min of decompression, residual deformation of the cord is observed. As inflammatory responses in the cord ensue, and the swelling cord fills the subarachnoid space, the cord pushes up against the dura within 5 hours of decompression (Jones et al., 2012).

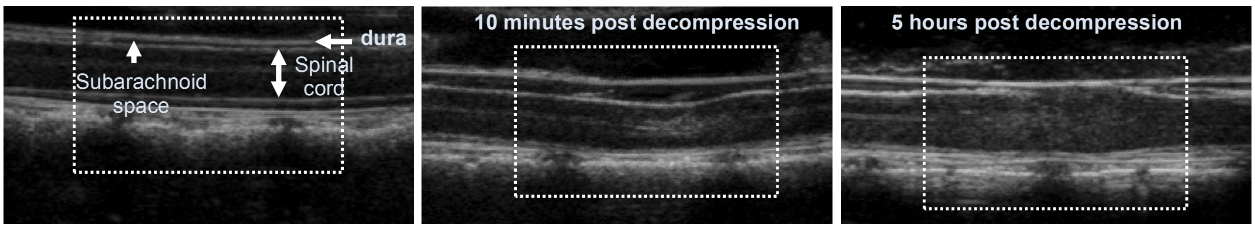
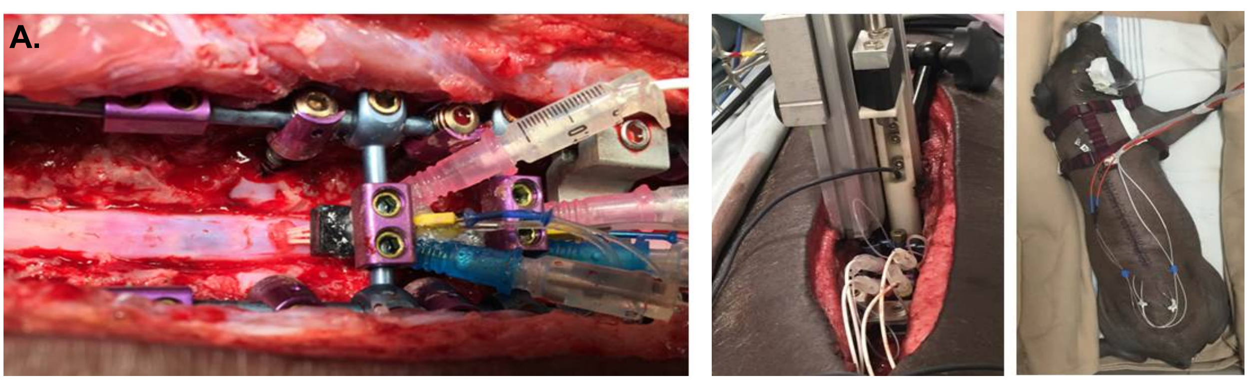
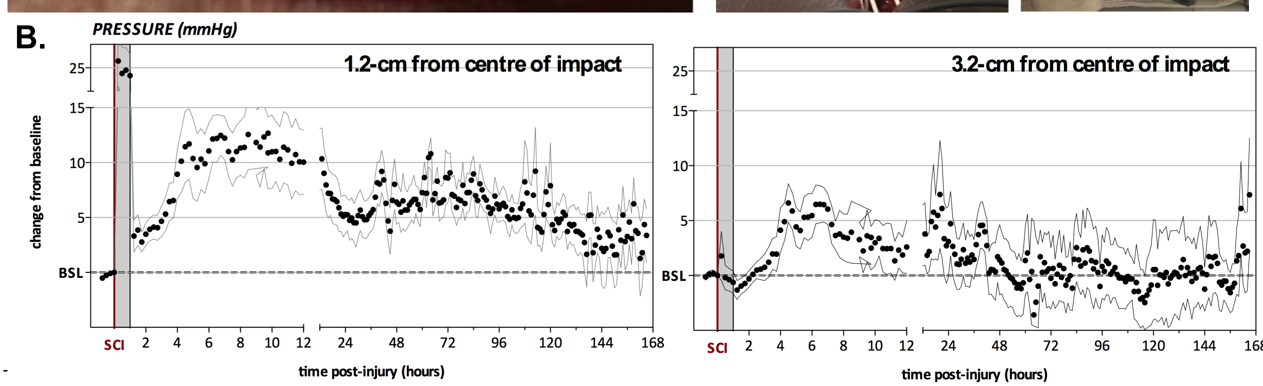


Figure 2: Monitoring of hydrostatic spinal cord pressure in acute SCI using a porcine model. (A) Surgical set-up to measure hydrostatic pressure within the spinal cord for 7 days. Three probes for monitoring oxygenation and blood flow, hydrostatic pressure and microdialysis were inserted through the dura and into the spinal cord 1.2 and 3.2 cm caudal to the injury. The recording wires from each probe were brought out of the surgical field, tunnelled through the skin and collected along the back of the animal. **(B)** At the injury site (1.2-cm) contusion injury followed by compression (grey shading) resulted in high intraparenchymal pressure. Following decompression intraparenchymal spinal cord pressure drops, after which it rises again remaining high for days, which suggests considerable post-SCI swelling. Such increases in cord pressure were also observed as far as 3.2-cm away from the trauma site.





3.1 Methods

In Year 1 of this award we examined whether duraplasty after acute thoracic spinal cord injury (SCI) in a porcine model could improve long-term functional outcome after injury. The T10 spinal cord was contused with a weight drop device (Figure 3) in which a 50g weight impact rod was dropped from a 20-cm height followed by 5 minutes compression (150g). Following injury, in n=6 animals a C6-T13 transverse dural incision was performed and a 10x1 cm artificial dural graft was sutured to the remaining dura mater in a watertight manner using a running suture of 6-0 Prolene, including the use of fibrin sealant to reinforce sutures (Figure 3). The duraplasty procedure took about 1-1.5 hours to complete. Control animals (n=6) received an identical spinal cord contusion, however without expansile duraplasty (for detailed information on the animals see Table 1).

Behavioral testing was performed weekly up to 12 weeks post-injury using the Porcine Thoracic Injury Behavior Scale (Figure 4), a 10-point scale, with 1-3 describing hindlimb movements without weight support and 4-10 describing varying increasing degrees of stepping and postural ability. Previously it was demonstrated that these behavioral scores of motor dysfunction correlate significantly with the extent of tissue sparing at the injury site (Lee et al., 2013).

Lower extremity somatosensory evoked potentials (SSEPs) of the posterior tibial nerve recordings were recorded to determine functional recovery (Figure 5). Posterior tibial nerve SSEPs assesses the integrity of somatosensory pathways by periodic percutaneous stimulation of the posterior tibial nerve using needle electrodes. These pathways ascend through structures including the lumber plexus, lumbosacral roots, ipsilateral gracile tract in the dorsal comlumn, ipsilateral gracile nucleus of the lower brain stem, and contralateral VPL nucleus of the thalamus before reaching the contralateral medial pariental cortex. To measure SSEPs, a total of 4 electrodes were placed 1 cm lateral to the sagittal suture and 1 cm anterior and posterior to the coronal suture. Steel screws 5/8 inches in length were inserted 5mm into the scull and connected to alligator clips.

Table 1. Experimental information and current status of the animals

ID	Species	SX date	Injury Specifics	Force (kdyn)	Weight (kg)	Treatment	Status
7753	Yucatan	20-Jul-15	**Contusion:** 50gr/20cm **Compression:** 150gr/5min	2950.00	21.5	duraplasty	Euthanized
7762	Yucatan	20-Jul-15		2409.00	20.5	SCI only	Euthanized
7731	Yucatan	21-Jul-15		2976.00	19.0	duraplasty	Euthanized
7721	Yucatan	21-Jul-15		2170.00	20.5	SCI only	Euthanized early
7732	Yucatan	22-Jul-15		2014.00	18.5	duraplasty	Euthanized
7743	Yucatan	22-Jul-15		3482.00	18.5	SCI only	Euthanized
7730	Yucatan	29-Jul-15		3132.00	19.5	duraplasty	Euthanized
7751	Yucatan	29-Jul-15		2935.00	20.0	SCI only	Euthanized
7736	Yucatan	4-Aug-15		2907.00	19.0	duraplasty	Week 12
7744	Yucatan	4-Aug-15		2681.00	19.5	SCI only	Week 12
7758	Yucatan	5-Aug-15		3047.00	21.5	duraplasty	Week 12
7759	Yucatan	5-Aug-15		2388.00	19.0	SCI only	Euthanized early
7800	Yucatan	19-Aug-15			25.0	SCI only	Week 10
7791	Yucatan	19-Aug-15		2145.00	24.0	SCI only	Week 10

Figure 3: Understanding the effect of relieving spinal pressure after SCI through duraplasty. (A) Following a T10 dorsal spinal contusion injury and **(B)** C6-T13 transverse dural incision, a 10x1 cm artificial dural graft (Medtronic, US) was sutured to the remaining dura mater and sealed with fibrin glue. Control animals received an identical spinal cord contusion, however without expansile duraplasty.

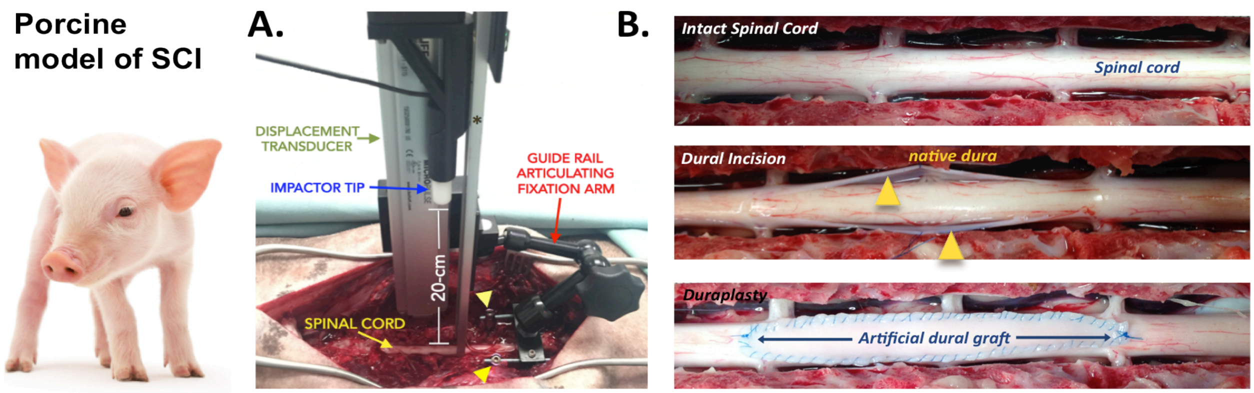
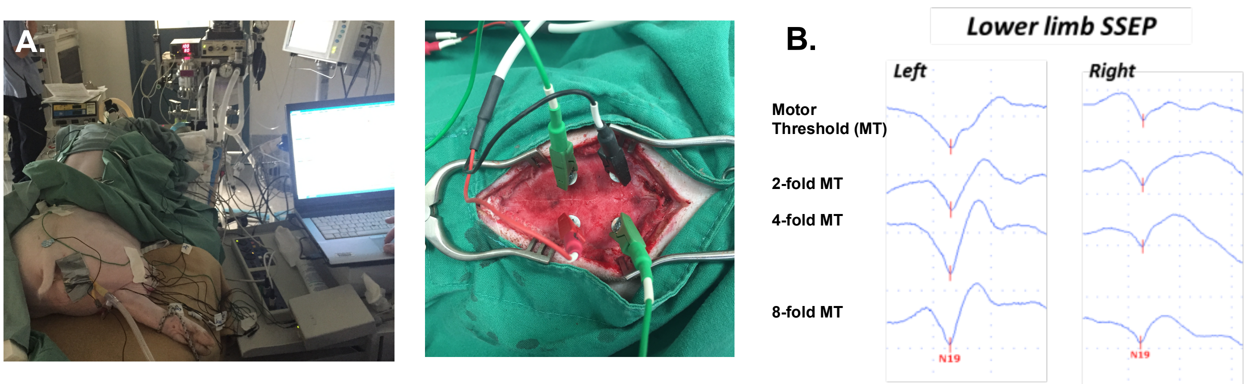


Figure 4: Porcine Thoracic Injury Behavior Scale. PTIBS is a 10-point scale, with 1-3 describing hindlimb movements without weight support and 4-10 describing varying increasing degrees of stepping and postural ability (Lee et al., 2013).

Score	Description
1	No active hindlimb movements, with rump and knees on the ground
2	Active hindlimb movements, with rump and knees on the ground
3	Active, hindlimb movements, with “weight-bearing extensions” that lift the rump and knee transiently off the ground (hip joints are flexed but knee joints flexed and extended)
4	Active rhythmic hindlimb crawling with at least 3 reciprocating gait cycles (Crawling: L-R-L-R-L-R). Rump off the ground constantly and transient “weight bearing extensions”.
5	The animal can take at least two steps (and up to six steps) with the rump and knee constantly off the ground during the steps. The knees do not fully extend. Hoof placement is a combination of dorsal and plantar. Balance while stepping is impaired.
6	The animal can take more than six steps with the rump and knee constantly off the ground. The knees do not fully extend. Hoof placement is a combination of dorsal and plantar. Balance while stepping is impaired.
7	The animal can take at least two steps (and up to six steps) with the knees fully extended. Hoof placement is a combination of dorsal and plantar. Balance while stepping (walking) is impaired.
8	The animal can take more than six steps with the knees fully extended. Hoof placement is a combination of dorsal and plantar. Balance while stepping (walking) is impaired.
9	The animal can take more than six steps with the knees fully extended. Hoof placement is planter. Trunk imbalanced as the animal steps (walks).
10	The animal demonstrates grossly normal ambulation, with normal balance.

Figure 5: Intraoperative somatosensory evoked potential monitoring (A) During the surgical procedure tibial SSEPs recordings were monitored bilaterally and made from the cortical electrodes in the hindlimb region contralateral to the stimulated hindlimb. For placement of the scalp electrodes, a 10-cm midline longitudinal incision was made at the level of the ears extending anteriorly towards the snout (Figure 1B). Dissection was carried to the level of the bone until the sagittal and coronal sutures were visualized. A total of 4 electrodes were placed 1 cm lateral to the sagittal suture and 1 cm anterior and posterior to the coronal suture. Because of the thickness of the pig's skull, and its high impedance to current flow, screws were placed into the skull. The skull was carefully drilled bicortically until the inner table of the skull was penetrated. Steel screws 5/8 inches in length were inserted and connected to alligator clips. The electrodes made very light contact with the dura mater, but did not compress the dura or brain structures. The tibial nerve was stimulated proximal to the ankle with subcutaneous needle electrodes. **(B)** Representative examples: bilateral lower limb SSEPs. For the SSEPs, we recorded a so-called recruitment curve: We stimulated with different intensities starting with the motor threshold (i.e., the intensity that elicits a 'hoof'-movement), 2 fold, 4 fold, and 8 fold the motor threshold.

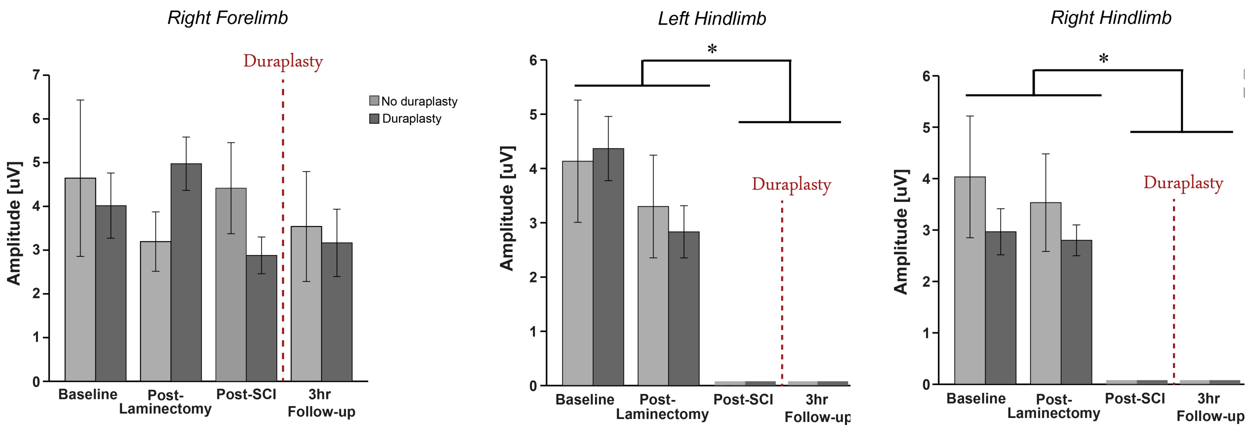


3.2 Results

SSEPs: Currently, SSEPs have been assessed for 4 different time points: Baseline (after screws are inserted in scull), after laminectomy, immediately after SCI and 3hrs post SCI. Since the injury was induced at T10, the SSEP signals from forelimbs' non-injured pathways were expected to stay constant at any time during the survival periods (Figure 6A). They were used for internal control purposes to ensure the quality of the recordings and the electrodes at each time point. A change in SSEP signals from forelimbs would indicate that other variants, apart from the desired injury, affected the SSEP recordings. No significant change was observed in the forelimb SSEP signals over time.

Hindlimb SSEPs showed no major change in SSEP amplitudes after laminectomy (Figure 6B). As it can be clearly seen, the hindlimb SSEP signals from both groups degraded significantly within minutes after SCI and did not recover during the following 3 hours, indicating complete interruption of spinal pathways. Comparing the SCI-control vs. SCI-duraplasty groups, there is no significant difference between the hindlimb SSEP amplitudes. Of course we didn't expect duraplasty to affect SSEPs that early after the procedure and for now merely confirms that injury severity is comparable between the two groups. Currently we are analyzing forelimb and hindlimb SSEPs for the 12-week time point.

Figure 6: Forelimb and hindlimb somatosensory evoked potentials (SSEPs). Note: Abolished lower limb SSEPs after SCI



PTIBS: Currently we analyzed the PTIBS recordings up to 6 weeks post-injury of all the animals. Our preliminary data suggest that duraplasty surgery improves functional recovery in the majority of the animals compared to SCI-animals without duraplasty (Figure 7). Animals in both the SC-control and SCI-duraplasty group demonstrated behavioral recovery over time. In our studies, most SCI-control animals (84%) showed hindlimb dragging (PTIBS score of 1-3) up to 5 weeks post-injury. However within the first week after SCI, half of the animals that received a duraplasty after SCI (50%) were already capable of weight-supported rhythmic hindlimb movements or making some steps (PTIBS ≥ 4). This improvement could not be explained by differences in impact force, displacement, velocity or bodyweight (Figure 8).

We hypothesis that the improved functional recovery following duraplasty surgery may be related to reduced spinal cord pressure which ultimately leads to attenuation of many facets of secondary damage. Currently we are conducting further studies evaluating the consequence of duraplasty on intraparenchymal spinal cord oxygenation, blood flow, and pressure, as well as, the downstream metabolic response using microdialysis. These data will further our understanding of the importance of relieving the ‘intrinsic’ pressure of the spinal cord to improve recovery after a traumatic SCI

Figure 7: Behavioural recovery is improved in the majority of the animals after duraplasty surgery. (Top) average PTIBS per group, (Bottom) each dot represents an individual animal. On each box, the central line marks the median, the edges of the box mark the 25th and 75th percentiles, and the whiskers mark the most extreme data points not considered outliers.

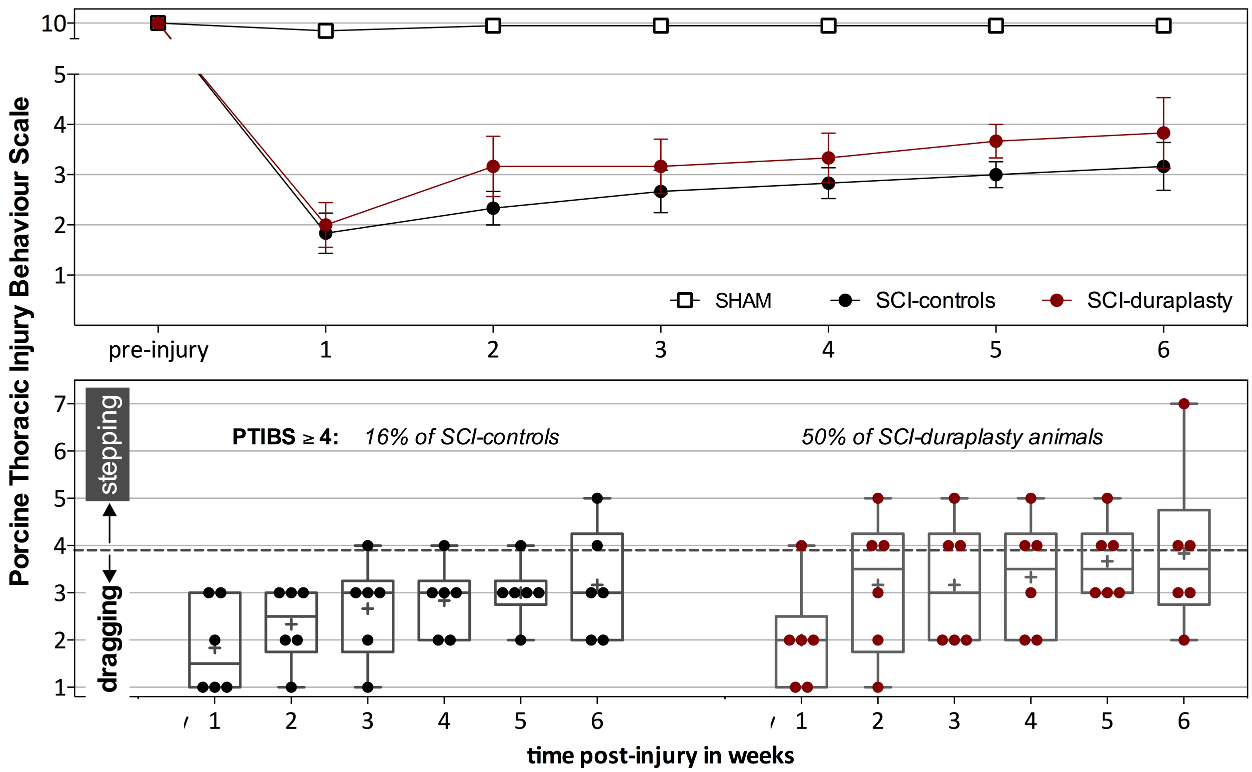
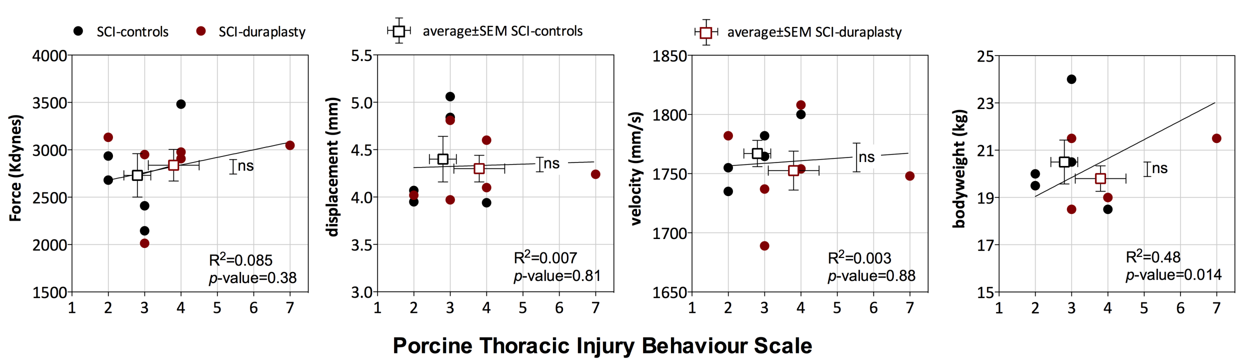


Figure 8: Correlation coefficient of PTIBS score and various injury parameters. No correlation was observed between the PTIBS at 6 weeks post-injury and the actual force, displacement or velocity at impact. Correlation analysis demonstrated a linear relationship between PTIBS and body weight after SCI, suggesting that animals with higher bodyweight at injury was shown to have higher PTIBS scores at 6 weeks post injury. However, no differences in bodyweight between the SCI-control and SCI-duraplasty group was observed.



4 KEY RESEARCH ACCOMPLISHMENTS

- Duraplasty surgery is feasible in our Porcine model of SCI
- Preliminary data suggest that duraplasty surgery improves functional recovery early after SCI compared to SCI-animals without duraplasty

5 CONCLUSION

Our preliminary data suggest that duraplasty surgery might improves functional recovery early after SCI. Performing a duraplasty to expand the space around the spinal cord would be a feasible neurosurgical procedure to add to the clinical spinal stabilization and decompression procedure, in the absence of other more novel neuro-regenerative treatments. In the next Year we will be conducting further studies evaluating the consequence of duraplasty on intraparenchymal spinal cord oxygenation, blood flow, and pressure, as well as, the downstream metabolic response using microdialysis. These data will further our understanding of the importance of relieving the 'intrinsic' pressure of the spinal cord to improve recovery after a traumatic SCI.

6 PUBLICATIONS, ABSTRACTS AND PRESENTATIONS

Poster presentation, Society for Neuroscience 2015, Chicago, Illinois, Oct 17-21:

Katelyn Shortt, Femke Streijger, Neda Manouchehri Kitty So, Elena Okon, Brian K. Kwon (2015) *The Effect of Duraplasty on Behavioral and Functional Recovery in Yucatan Mini-Pigs After Traumatic SCI.*

7 INVENTIONS, PATENTS AND LICENSES

Nothing to report

8 REPORTABLE OUTCOMES

Nothing to report

9 OTHER ACHIEVEMENTS

Nothing to report

10 REFERENCES

Werndle MC, Saadoun S, Phang I, Czosnyka M, Varsos GV, Czosnyka ZH, Smielewski P, Jamous A, Bell BA, Zoumprouli A, Papadopoulos MC (2014) ***Monitoring of spinal cord perfusion pressure in acute spinal cord injury: initial findings of the injured spinal cord pressure evaluation study.*** Critical Care Medicine; 42(3): 646-55. PMID: 24231762.

Jones CF, Cripton PA, Kwon BK (2012) ***Gross morphological changes of the spinal cord immediately after surgical decompression in a large animal model of traumatic spinal cord injury.*** Spine (Phila Pa 1976); 37(15): E890-9. PMID: 22433504.

Lee JH, Jones CF, Okon EB, Anderson L, Tigchelaar S, Kooner P, Godbey T, Chua B, Gray G, Hildebrandt R, Cripton P, Tetzlaff W, Kwon BK. (2013) ***A novel porcine model of traumatic thoracic spinal cord injury.*** J Neurotrauma; 30(3): 142-59. PMID: 23316955.

11 APPENDICES

None